Supporting Information

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SI Text

Cloning, Expression, and Purification. A gene encoding *A. thaliana* (6-4) photolyase (UVR3) was isolated from cDNA (kindly provided by Tokitaka Oyama of Kyoto University), by using the 5′ region of the UVR3 gene amplified from the library by PCR as a probe. To avoid mutation, a short 5′ EcoRI/Dra fragment amplified by PCR with EcoRI tag and a Dra/PstI fragment derived from the library were inserted between EcoRI and Pst sites of pKK223 (Amersham Pharmacia). To express protein, the plasmid carrying UVR3 gene was transformed into *E. coli* JM 109, which were cultured at 25 °C, induced by 1 mM isopropyl β-D-thiogalactoside and harvested after 14–16 h. *At*64PHR was purified with Blue Sepharose, DNA cellulose, hydroxyl apatite, and monoS column chromatography. The protein was stored at −80°C in a solution containing 50 mM Tris·HCl (pH 8.0), 50 mM NaCl, 1 mM DTT, and 50% glycerol.

For mutagenesis, the isolated gene was inserted into pGEX-4T-1 (Amersham Pharmacia) containing the GST gene. Mutant clones were constructed by using the QuikChange Site-Directed Mutagenesis Kit (Strategene). Gene products were purified as described (1). Repair activity of the (6-4) photolyase mutants was tested on dsDNA substrates containing (6-4) photoproducts as described (2, 3).

Fluorescent DNA Distortion Assay. Oligonucleotides containing the (6-4) photoproduct (PP) or normal TT, d(CCTACGCAAAT-XX-GGCATCC) (XX = PP or TT), were hybridized to a complementary strand containing 2-aminopurine (Ap), d(G-GATGCC-Ap-AATTTGCGTAGG) as described (4). These duplexes were dissolved at 0.3 μM in a buffer containing 50 mM Tris·HCl (pH 8.0), 100 mM NaCl, 1 mM 2-mercaptoethanol, and 25% glycerol, and fluorescence spectra were measured at 10 °C, in the absence or presence of 0.35 μM WT *At*64PHR or Arg-420 mutant. The excitation wavelength was set to 313 nm.

Crystallization, Data Collection, and Structure Determination. The *At*64PHR buffer was replaced with 50 mM Tris·HCl (pH 8.0), 50 mM NaCl, 10% glycerol, and 10 mM DTT. Crystals of *At*64PHR were obtained at 4 °C by hanging drop vapor diffusion against 100 mM Hepes (pH 6.6), 25 mM potassium acetate, and 20% polyethylene glycol 6000. Drops contained 1 μL of each protein (>25 mg/mL) and well solution, plus 0.2 μL of 30% MPD. Crystals were flash-frozen with mother liquor plus 10–15% ethylene glycol under liquid nitrogen. X-ray diffraction data were collected at the Advanced Light Source beamline 8.3.1 and processed with DENZO/Scalepack (5).

We determined the structure by molecular replacement, using diffraction data from 10- to 4-Å resolution. The search probe consisted of 4 overlaid structures: a partial theoretical model for Xl64PHR (3), and crystal structures for *E. coli* CPD photolyase (PDB ID Code 1DNP), cyanobacterial CPD photolyase from *Anacystis nidulans* (PDB ID Code 1QNF), and the cyanobacterial cryptochrome DASH from *Synechocystis* sp. PCC6803 (PDB ID Code 1NP7). In the crystal lattice, *At*64PHR molecules are linked into chains by intermolecular salt bridges joining conserved Arg-420 with Glu-211. Initial phases obtained with AmoRe (6) gave a correlation factor of 27.6% and an *R* factor of 48.6% in the resolution range of 10 to 4 Å. The *At*64PHR model was fit manually with TURBO FRODO (7) and refined with CNS (8). Data collection and refinement statistics are summarized in Table S1.

Transcription Assay. 293T cells were reverse-transfected in 96-well plates as described (9). Briefly, for each transfection we prepared a plasmid DNA mixture consisting of 25 ng of Per1-Luciferase reporter construct, 50 ng CMV-mBMAL1, 120 ng CMV-hCLOCK, CMV-CRY (0–5 ng, as indicated) plus filler DNA to bring the total to 250 ng. The DNA mix was diluted in a final volume of 50 μL of serum-free DMEM containing 0.75 μL of Fugene 6. The Fugene/DNA/serum-free DMEM mixes were then distributed into a 96-well, white, flat-bottom tissue culture plate and incubated at room temperature for 20–40 min. After this period, 50 μL of a 293T cell suspension, in 20% FBS-containing DMEM (800,000 cells/mL; 40,000 cells per well), were distributed onto each well and the plate was subsequently placed in a standard tissue culture incubator. Twenty-four hours after transfection, cell extracts were prepared with BriteGlo reagent (Promega) according to the manufacturer's instructions and the luciferase activity was measured in a TECAN M200 luminometer.

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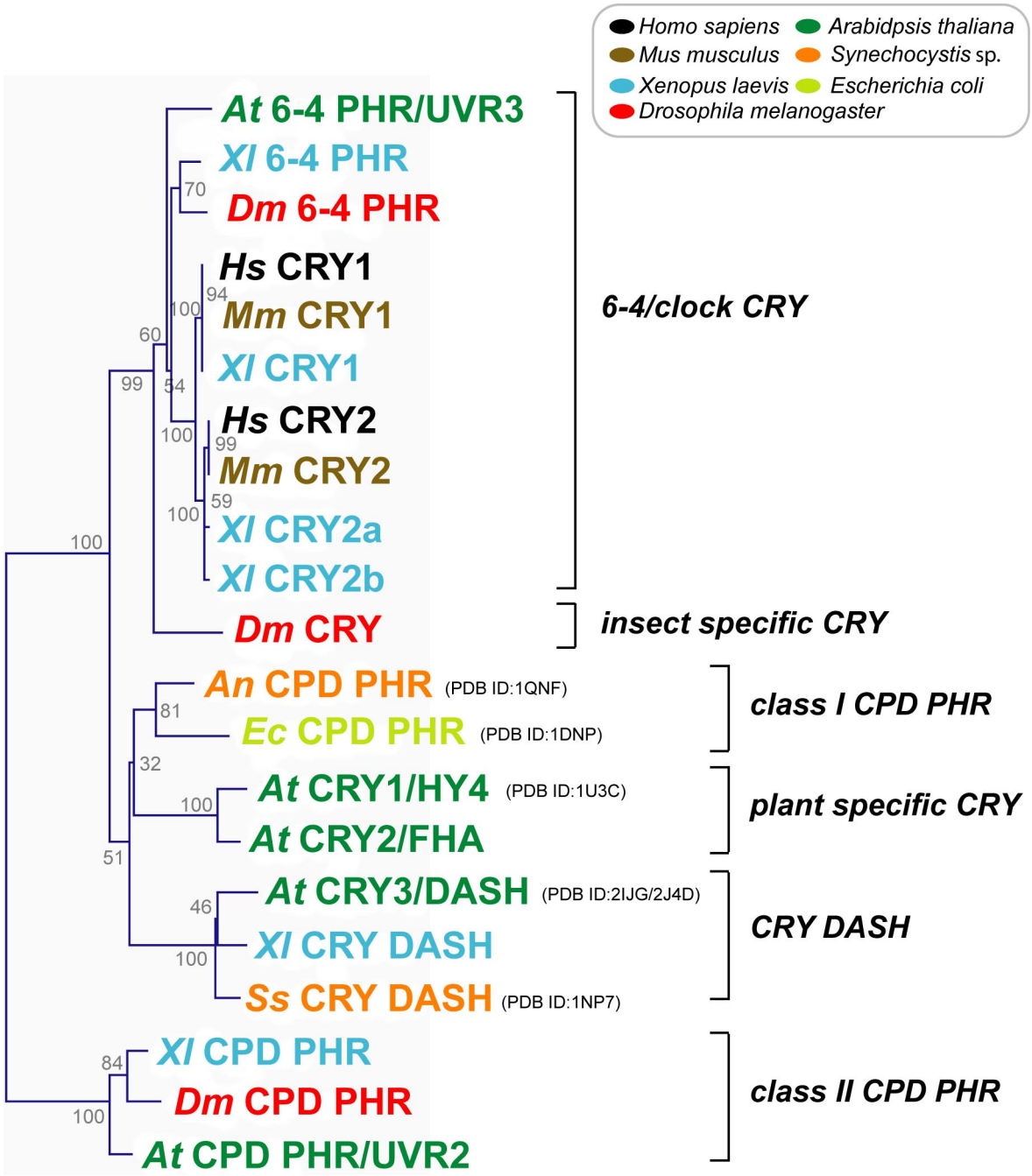

Fig. S1. Phylogenetic tree for PHR/CRY family has 4 distinct CRY clusters: 6-4/clock, insect-specific, plant-specific, and CRY DASH (found in plants, animals and bacteria). The (6-4) photolyases belong to the 6-4/clock cluster, and class I CPD photolyases resemble plant-specific CRYs, but the class II CPD photolyases cluster alone.

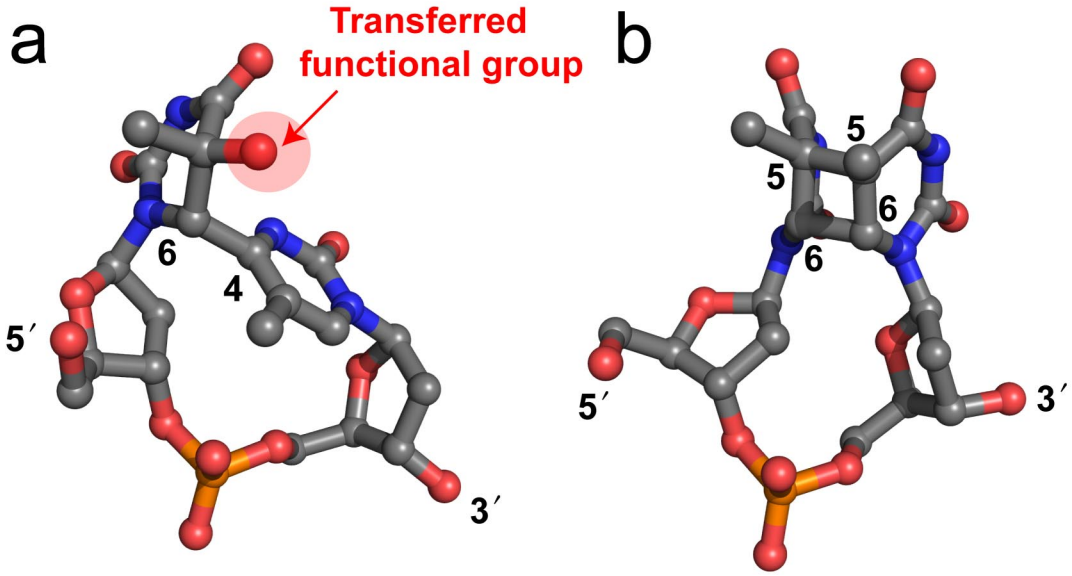

Fig. S2. Structure of UV-induced photoproducts. (*a*) Pyrimidine (6-4) pyrimidone photoproduct (PDB ID code 1EHL). (*b*) Cyclobutane pyrimidine dimer (PDB ID code 1VAS).

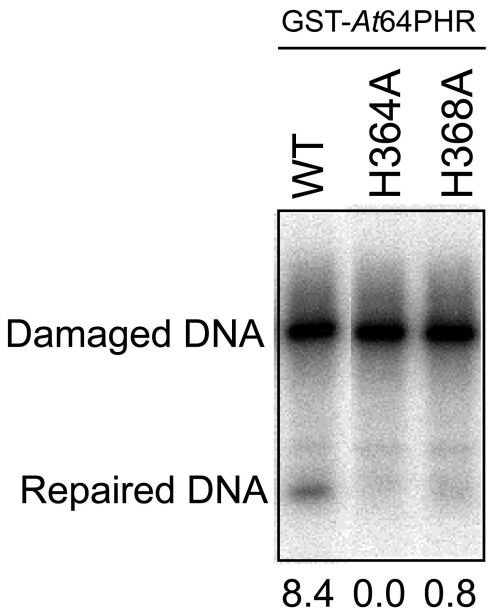

Fig. S3. Gel showing loss of DNA repair activity for single-site *At*64PHR mutants in which active-site His-364 and His-368 have been substituted with Ala.

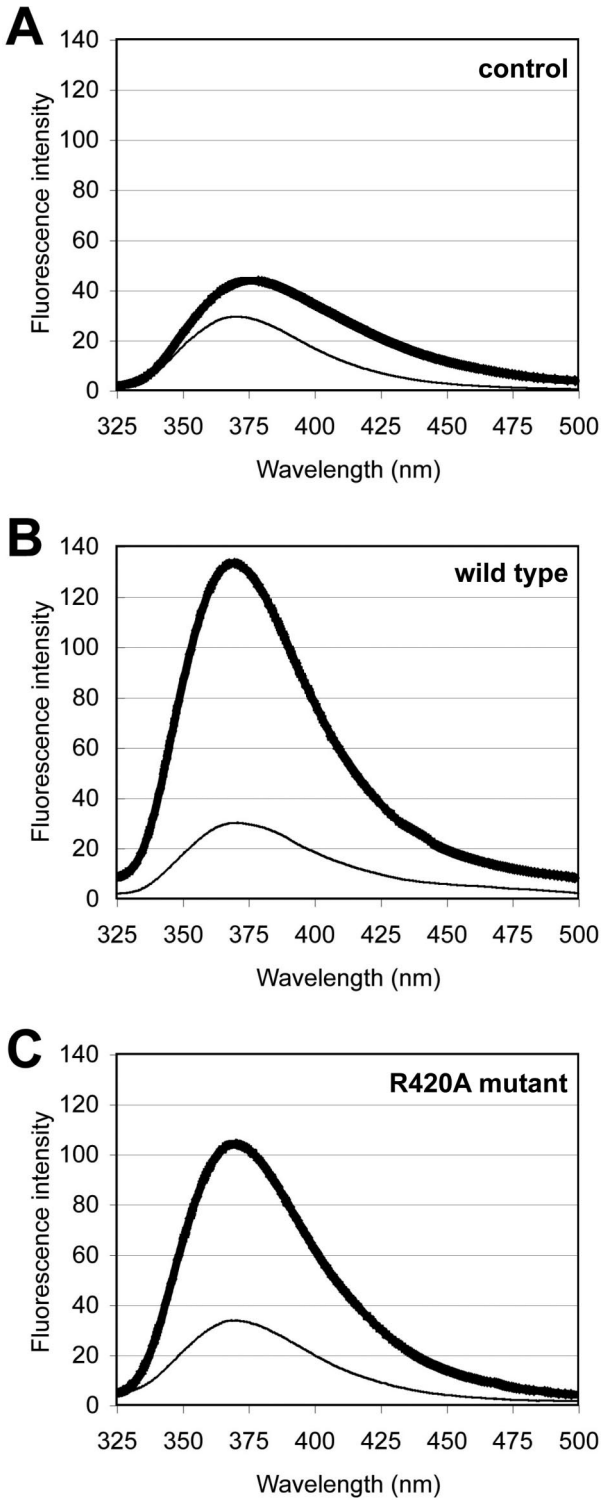

Fig. S4. Fluorescence detection of (6-4) photoproduct flipping out of duplex DNA by WT and mutant *At*64PHR. Substitution of active-site Arg-420 (His in clock CRYs) with Ala does not abolish photoproduct recognition.

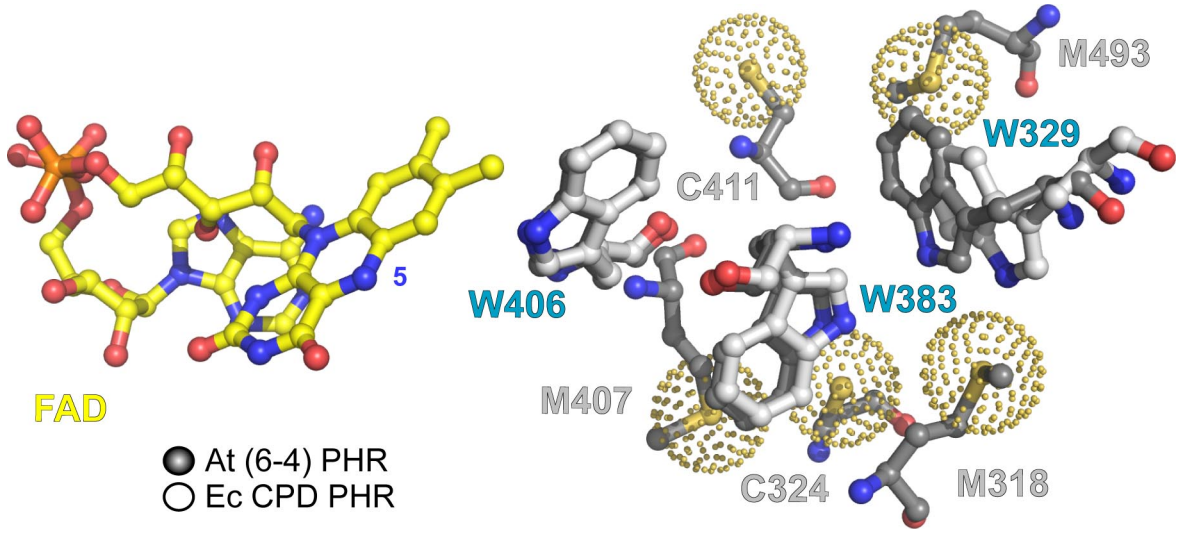

Fig. S5. The *At*64PHR Trp electron-transfer pathway (gray) to FAD (yellow) is conserved structurally with the Trp triad of *E. coli* CPD photolyase (white), but exhibits some modifications. The middle Trp-383 and outer Trp-329 side chains hydrogen-bond to sulfur atoms (spheres) contributed by Met-318 and Cys-324 of the sulfur loop. Three additional sulfur atoms cluster nearby.

Table S1. Crystallographic data and refinement statistics

Measurement	Value
X-ray data	
Space group	$P2_12_12_1$
	a = 112.4 Å
	b = 139.0 Å
	c = 143.1 Å
	$\alpha=\beta=\gamma=90.0°$
Resolution, Å	60–2.7
Total no. reflections	917,413
Unique reflections	62,218
No. reflections used	59,584
Completeness, %	99.8 (98.4)
Average I/δ	21.9 (3.1)
R_{sym}	0.091 (0.446)
Refinement	
Resolution, Å	60–2.7
No. protein atoms	12,553
No. water atoms	678
R_{work}, %	20.2
R_{free} %	23.8
Bond length, Å	0.0067
Bond angles, °	1.29
Favored, %	92.6
Allowed, %	98.4

Numbers in parentheses are for the highest-resolution shell (2.8–2.7 Å) of the data. $R_{sym} = \Sigma_h \Sigma_i |\bar{I}(h) - I_i(h)| / \Sigma_h \Sigma_i I_i(h)$, where $I_i(h)$ is the ith measurement of the intensity for Miller indices h, and $I(h)$ represents the mean intensity value of the symmetry (or Friedel) equivalent reflections of Miller indices h. $R_{work} = \Sigma_h ||F_o| - |F_o|| / \Sigma_h |F_o|$. The formula for R_{free} is the same as that for R_{work}, except that it is calculated with 5% of the structure factors that had not been used for refinement.